Excited State Trapping and the Stepanov Relation with Reference to Photosystem I

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ABSTRACT It has been previously demonstrated that the Stepanov equation provides a rather good description of the absorption/fluorescence spectra in Photosystem I, even though excited state equilibration is not rapid with respect to the excited state decay. In the present article this apparent contradiction is examined analytically for two-state systems and numerically for many-state systems. It is demonstrated that, in the special case of the trapping process being associated with the initially populated state, neither very rapid excited state equilibration nor a transfer equilibrium, which approximates a true Boltzmann distribution, are prerequisites to obtaining a very close approximation to a correct Stepanov result. This interesting conclusion is discussed in terms of plant Photosystem I (PSI-200). It is concluded that whereas, in compartmental modeling, photochemical trapping may be formally associated with the bulk antenna pigments due to the strong energy coupling between them and the trap pigments, this is not the case for the red spectral forms.

INTRODUCTION

The relation between the steady-state electronic absorption and fluorescence emission spectra of a dye solution, under conditions of complete vibrational thermalization of the excited state, is given by the so-called Stepanov expression (Eq. 1) which derives from theoretical considerations made initially by Kennard (1918), and subsequently, in a more concise way, by Stepanov (1957) as

$$F_\nu = D(T)A_\nu\nu^2 e^{-h\nu/kT}, \quad (1)$$

where F_ν is the fluorescence spectrum, A_ν is the absorption spectrum, h is the Planck constant, and k is the Boltzmann constant. $D(T)$ is a temperature-dependent term that is independent of the frequency. Its precise meaning is given in Stepanov (1957). This equation, which explicitly excludes pre-equilibration emission, is readily extended to pigment clusters in the assumption that thermalization within and between the excited state pigment manifolds is very rapid with respect to the excited state decay (Knox and Van Metter, 1979; Zucchelli et al., 1995; Croce et al., 1996; Dau, 1996; Dau and Sauer, 1996; Pålsson et al., 1998; Cometta et al., 2000). Here we will refer to this as very rapid equilibration (VRE). We wish to underline that VRE is not in any way an absolute parameter but has the meaning of being very rapid with respect to the excited state decay of the system. This consideration is important when we examine systems in which a photochemical trap is present. In the case of pigment clusters, the equation has exactly the same form as for a dye solution, as the excited state vibrational manifolds of the separate pigments are treated essentially as though they were associated with a single molecular entity. Within the absorption/fluorescence overlap interval Eq. 1 usually works well for isolated chlorophyll/protein complexes, with small deviations being attributed either to incomplete equilibration between vibrational manifolds (Knox et al., 1999; Knox and Marshall, 2000), particle heterogeneity (van Metter and Knox, 1976), uncoupled pigments (Zucchelli et al., 1995), or the presence of reaction center trapping (Croce et al., 1996; Jennings et al., 1997). Probably the most remarkable example of a good Stepanov fit in photosynthesis concerns isolated Photosystem I complexes, where the main (antenna) absorption band, associated with the lowest electronic excited state (Q_y), is maximal at 680 nm and with a weakly absorbing red tail extending out to ~750 nm. In this case, Eq. 1 exactly describes the experimental emission maximum near 735 nm and also closely describes the main emission band shape, for room temperature measurements with both higher plant PSI-200 and cyanobacterial Photosystem I (PSI) core complexes (Croce et al., 1996; Pålsson et al., 1998; Cometta et al., 2000). In fact, the Stepanov behavior for PSI at room temperature is considerably more accurate than for isolated chl *a* in a variety of solvents (Szalay et al., 1974; van Metter and Knox, 1976). It therefore turned out to be somewhat surprising when a subsequent time-resolved fluorescence study (Croce et al., 2000), using the same PSI preparation as in the earlier Stepanov analysis, demonstrated unequivocally that VRE does not occur in PSI. In fact, from analysis of the first spectral moment during the fluorescence decay, it was shown that spectral equilibration is never attained, with spectral evolution displaying similar dynamics to the decay process itself. This slow spectral evolution specifically regards the low energy forms and clearly violates the main assumption on which the Kennard-Stepanov theory is based for pigment clusters. In the present analysis this point is investigated for pigment systems in which an excited state trap is present. It is demonstrated that, in the special case of

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the trapping process being associated with the initially populated state, VRE is not a prerequisite to obtaining a very close approximation to a correct Stepanov result. This interesting and novel insight is discussed in terms of plant Photosystem I (PSI-200).

RESULTS AND DISCUSSION

We will initially consider a coupled two-state kinetic system as represented in Fig. 1. With this simple model, for which analytical solutions are available for both the eigenvalues and eigenvectors, it is possible to clearly illustrate the essential points.

In the model of Fig. 1 the lowermost energy level is the ground state and the upper levels represent the vibrational levels of the excited states A and B. It is assumed that VRE occurs within each of the two vibrational manifolds of the excited states and that the fluorescence associated with each of them may be described by the Stepanov expression (Eq. 1). Excited state transfer between the two states is possible with rate constants k_1 and k_{-1}, where the ratio k_1/k_{-1} may be approximated by the Boltzmann expression (detailed energy balance) considering the lowest ground and excited state levels (0,0 transitions) of each of the two states. This is the mirror symmetry axis for the absorption/fluorescence bands. Excited state relaxation occurs via the rate processes k_2 and k_3 and excited state trapping occurs from the A state by k_4 and from the B state by k_5. The fluorescence spectrum at any time, t, is given by

$$F_\nu(t) = D^{\mathrm{A}}(T)\rho^{\mathrm{A}}(t)A_\nu^{\mathrm{A}}\nu^2 e^{-\mathrm{h}\nu/\mathrm{kT}} + D^{\mathrm{B}}(T)\rho^{\mathrm{B}}(t)A_\nu^{\mathrm{B}}\nu^2 e^{-\mathrm{h}\nu/\mathrm{kT}}, \tag{2}$$

where $\rho^{\mathrm{A}}(t)$ and $\rho^{\mathrm{B}}(t)$ are the time dependent probability terms for excited state population in states A and B, respectively. Thus, Eq. 2 describes the time-resolved fluorescence band shape, during a fluorescence lifetime experiment, for this two-state system. It should be emphasized that it is the excited state population probability terms ($\rho^{\mathrm{A,B}}$) which determine the Stepanov behavior of this two-state system, as exact Stepanov behavior for each state is assumed.

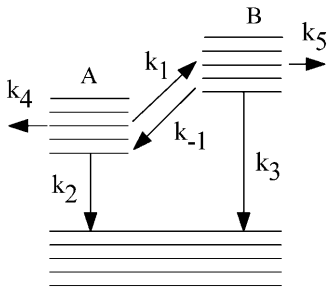

FIGURE 1 Kinetic scheme for a two-state pigment system in which the ground state and the lowest lying excited state manifolds are schematically represented. Excited state transfer between the pigment excited states occurs via k_1 and k_{-1}. Relaxation to the ground state occurs via k_2 and k_3, and excited state trapping via k_4 and k_5.

In this paragraph we develop some simple expressions which justify the use of Kennard-Stepanov theory for pigment clusters without a trap. The set of differential equations for the time-dependent excitation probabilities ($\rho^{\mathrm{A,B}}$) of the two-state system are $d\rho^{\mathrm{A}}/\mathrm{dt} = k_{-1}\rho^{\mathrm{B}} - (k_1 + k_2 + k_4)\rho^{\mathrm{A}}$ and $d\rho^{\mathrm{B}}/\mathrm{dt} = k_1\rho^{\mathrm{A}} - (k_{-1} + k_3 + k_5)\rho^{\mathrm{B}}$. For the trapless situation $k_{4,5}$ are set to zero and we assume that only one of the two excited states is initially populated (state A) with VRE between the two excited state manifolds. Under these conditions $k_1, k_{-1} \gg k_2, k_3$, and the two excited states rapidly proceed to transfer equilibrium (Laible et al., 1994) with the excitation probabilities $\rho^{\mathrm{A,B}}(t)$ attaining the transfer equilibrium values of

$$\begin{aligned}\rho_{\mathrm{te}}^{\mathrm{A}} &= (2k_{-1} + k_3)/(2k_{-1} + 2k_1 + k_2 + k_3)\\ \rho_{\mathrm{te}}^{\mathrm{B}} &= (2k_1 + k_2)/(2k_{-1} + 2k_1 + k_2 + k_3).\end{aligned} \tag{3}$$

At transfer equilibrium $\rho_{\mathrm{te}}^{\mathrm{B}}/\rho_{\mathrm{te}}^{\mathrm{A}} = (2k_1 + k_2)/(2k_{-1} + k_3)$ and, for the condition of VRE between excited state manifolds, this is well approximated by the Boltzmann ratio k_1/k_{-1}. Under these conditions it is generally assumed that the fluorescence arising from pre-equilibrium states may be neglected and thus the steady-state absorption/fluorescence spectra are approximated by Kennard-Stepanov theory. This is essentially the situation which has been assumed to apply for chlorophyll-protein complexes and isolated photosystems (Knox and Van Metter, 1979; Zucchelli et al., 1995; Croce et al., 1996; Dau, 1996; Dau and Sauer, 1996; Pålsson et al., 1998; Cometta et al., 2000). Of course, in real steady-state measurements, the pre-equilibrium fluorescence is in fact measured. However, from the differential equations for the two-state system, written above, it may be shown that the time-integrated probability ratio is given by $\int \rho^{\mathrm{B}}(t)\mathrm{dt}/\int \rho^{\mathrm{A}}(t)\mathrm{dt} = k_1/(k_{-1} + k_3) \approx k_1/k_{-1}$, and is still therefore approximated by the Boltzmann ratio for VRE and hence, from Eq. 2, Kennard-Stepanov theory is applicable.

We now consider the case in which k_4 or k_5 are included and, as above, assume that only one of the two excited states is initially populated (state A). This is to be understood as the introduction of a significantly fast excited state trapping process, which may perturb the equilibrium ratio of ρ^{A} and ρ^{B} and hence lead to deviations from Kennard-Stepanov behavior. The transfer equilibrium population probabilities now become

$$\begin{aligned}\rho_{\mathrm{te}}^{\mathrm{A}} &= (2k_{-1} + k_3 + k_5)/(2k_{-1} + 2k_1 + k_2 + k_3 + k_4 + k_5)\\ \rho_{\mathrm{te}}^{\mathrm{B}} &= (2k_1 + k_2 + k_4)/(2k_{-1} + 2k_1 + k_2 + k_3 + k_4 + k_5),\end{aligned} \tag{4}$$

and from the differential equations for the two-state system, written above, it may be shown that the time-integrated probability ratio is given by

TABLE 1

Rate matrix (ns^{-1})						
	1	**2**	**3**	**4**	**5**	**6**
1	0.5	1200	0	103	38	12
2	120	0.5	3540	0	0	0
3	0	2000	2000	0	0	0
4	140	0	0	0.5	0	0
5	30	0	0	0	0.5	0
6	15	0	0	0	0	0.5

$$\int \rho^{B}(t)dt \Big/ \int \rho^{A}(t)dt = k_1/(k_{-1} + k_3 + k_5). \qquad (5)$$

As it is well known from compartmental photosystem modeling (e.g., Jennings et al., 1997, 2000; Byrdin et al., 2000; Gobets et al., 2001) that the photochemical trapping rate constants are of the same order of magnitude as the apparent rate constants for excited state transfer dynamics between pigment clusters, it is clear from Eq. 4 that, when trapping is from either the *A* state (k_4) or the *B* state (k_5), the $\rho^{B}_{te}/\rho^{A}_{te}$ ratio, which determines the transfer equilibrium in the presence of trapping, is not approximated by the Boltzmann ratio k_1/k_{-1}, due to the non-negligible value of k_4 or k_5. The same applies to the time integrated ratio when trapping is associated with the *B* state (Eq. 5). However, when trapping is associated with the initially populated *A* state (k_4), from Eq. 5 we have $\int \rho^{B}_{(t)}dt / \int \rho^{A}_{(t)}dt = k_1/(k_{-1} + k_3) \approx k_1/k_{-1}$, and Kennard-Stepanov theory will provide a close approximation for the absorption/fluorescence spectra (Eq. 2). Furthermore, this close approximation to Stepanov behavior is not dependent on VRE as there is no requirement that $k_{1,-1} > k_4$. This interesting result means that when trapping is associated with the same pigment cluster as that which is initially populated electronically, even when the transfer equilibrium distribution is clearly different from a Boltzmann distribution and VRE is absent, the time-integrated fluorescence closely approximates a Boltzmann distribution and hence a reasonable Stepanov behavior will be found. It is as if trapping by the absorbing pool were effectively reducing excitation of that pool.

The above considerations show that for a two-state system in which trapping occurs from the initially populated state, good Stepanov behavior does not require VRE between pigment clusters. It is important to know whether this conclusion also applies to many-state systems, as most photosystem modeling involves more than two states. As analytical solutions for many-state systems are not available, we have investigated this numerically and find that this conclusion is completely general and independent of the number of states with the condition that the transfer rates between the model compartments are at least 20× greater than the trivial relaxation processes (data not shown).

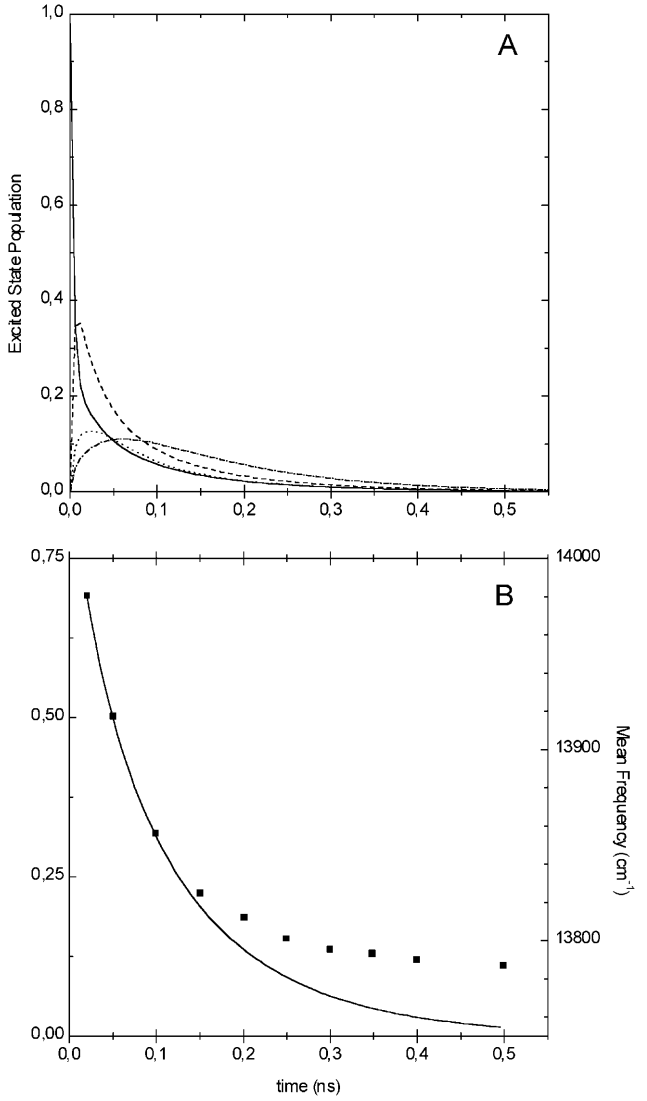

FIGURE 2 Model calculation for the four-state compartmental model of Photosystem I. The model is described in the text where the rate values are given (see Table 1, Rate Matrix). Trapping is associated with the bulk antenna and has the "effective" rate value of 45 ns^{-1}. Initial population is in level 1 (bulk antenna). (*A*) Population dynamics (*solid line*, bulk antenna; *dashed line*, red antenna form at 712 nm, i.e., level 4; *dotted line*, red form at 722 nm, i.e., level 5; and *dot-dashed line*, red form at 734 nm, i.e., level 6). (*B*) Overall excited state decay (*solid line*) and spectral evolution dynamics of the first central moment (*dots*). The first central moment (cm^{-1}) was calculated assuming Gaussian lineshapes of equal half-width for each of the four antenna levels.

In the following we shall specifically discuss the relevance of these findings to Photosystem I (see Introduction) and use, as a basis for this discussion, the compartmental model proposed by Croce et al. (2000) describing excited state decay in this photosystem. This model, represented as a six-energy level matrix, approximately describes the measurable fluorescence decay components (see Table 1, Rate Matrix, below).

TABLE 2

Antenna state	1	2	3	4	5
Bulk	0.231	0.227	0.239	0.241	0.263
Red forms 712 nm	0.313	0.310	0.289*	0.326	0.356
Red forms 722 nm	0.180	0.180	0.186	0.146*	0.205
Red forms 734 nm	0.275	0.282	0.285	0.146	0.176*

The time-integrated population probabilities ($\int\rho(t)$dt) for the four antenna compartments in the PSI-200 model (Croce et al., 2000) in which trapping is associated with the bulk antenna with a rate constant of 45.08 ns^{-1} (*column 1*; see text for explanation) compared with the Boltzmann factors for each antenna state (*column 2*). Also given (*columns 3–5*) are the relevant population probabilities when trapping is associated with each of the red absorbing states with rate constants between 9.7 and 12.5 ns^{-1}.

*The specific red form to which trapping is associated in the model calculations; for further explanations, see text.

Bold numbers indicate the energy levels.

Level 1 is taken as the mean energy of the bulk antenna (685 nm, 180-fold degenerate).

Level 2 represents the group of six inner core chls strongly coupled to P700 which have a collective absorption maximum at 695 nm (Croce et al., 1996; Jennings et al., 1997) and is sixfold degenerate.

Level 3 represents the P700 dimer (700 nm, twofold degenerate).

Levels 4, 5, and 6 represent the three red forms (712 nm, eightfold degenerate; 722 nm, twofold degenerate; and 734 nm, onefold degenerate).

The transfer direction is from the horizontal line of numbered levels toward the vertical line of numbered levels. The diagonal elements are the trivial excited state decay rates except for (3,3), which is the primary photochemical rate.

All energy levels are defined in terms of the mean wavelength position of the absorption bands and the ratio of each pair of rate processes is given by the population-weighted Boltzmann factor. The red forms are not directly coupled either with each other or with the inner core molecules.

This system has six eigenvalues, representing the decay lifetimes. The first two are on a femtosecond timescale and, as such, are not experimentally relevant. The third and fourth (3 ps and 19 ps) are too close to be readily resolved experimentally in the single photon counting measurements. The fifth and sixth (50 ps and 130 ps) are close to the experimental values for the main decay components.

In the experiments that this model describes, excitation was at 670 nm and therefore may be considered to be entirely in the bulk antenna chlorophyll (state 1). Trapping, however, can be seen to occur at the level of P700, which is not initially excited. Thus, at first sight, this situation seems different from that in which trapping occurs from the initially populated state. However, it should be noted that coupling between P700 and the inner core chlorophylls ($k_{2,3}$, $k_{3,2}$) is extremely strong and that between the inner core and the bulk ($k_{1,2}$, $k_{2,1}$) is also strong. Thus it is possible to associate the trapping process with the inner core and then with the bulk antenna by means of the equation

$$k_{\mathrm{tr}} = k_1k_2/(k_{-1} + k_2)$$

for systems of the kind

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B \xrightarrow{k_2}.$$

In this way, the low fluorescing inner core and P700 states are eliminated and the trapping constant, directly associated with the bulk antenna, becomes an ''effective'' trapping constant and has the value of 45 ns^{-1}. Elimination of these states leads to a minor decrease in the fluorescence yield (−2.5%) with respect to the complete model and the values for $\int\rho_{\mathrm{n}}(t)$ dt, $n = 1, 4, 5, 6$, are unchanged (data not shown). Thus, from the point of view of the fluorescence yield of the bulk chlorophylls, as well as the red forms, trapping, with the appropriate compartmental model-based rate constant, may be associated with the initially excited state (state 1). The results of this model calculation are shown in Fig. 2 *A*, for the excited state dynamics of states 1 (bulk); 4 (red forms, 712 nm); 5 (red forms, 722 nm); and 6 (red forms, 734 nm). To determine the dynamics of spectral evolution we have made the assumption that the emission of each of the four antenna states may be approximated by a Gaussian of similar bandwidth, and calculated the first central moment of the spectral distribution as a function of the decay time. In this way, Fig. 2 *B* shows the dynamics of spectral evolution, together with the overall excited state decay. It is clear that significant spectral evolution occurs for up to 0.3 ns, when >95% of the excited state population has decayed. These model calculations thus describe the experimental observation of Croce et al. (2000) for PSI-200, in which excited state equilibration is demonstrated to be slow. Table 2 shows the values for $\int\rho_{\mathrm{n}}(t)$dt, $n = 1,4,5,6$, together with those for the relative Boltzmann population values. It is clear that agreement between the two sets of data is very close. Thus from Eq. 2 good Stepanov behavior is expected, as has been experimentally demonstrated, and this occurs in the absence of VRE between the pigment compartments.

It should be mentioned that there are several suggestions in the literature that slow, rate limiting, energy transfer processes occur within the antenna of Photosystem II (Jennings et al., 1996, 2000; Vasil'ev et al., 2001) even

though it is known that Kennard-Stepanov theory accurately describes the absorption/fluorescence characteristics of this photosystem (Dau and Sauer, 1996). This may be explained by the very similar spectral characteristics of the PSII antenna pigment clusters (Jennings et al., 1993), which would render time-resolved spectral discrimination experimentally impossible (Jennings et al., 1996; Vasil'ev et al., 2001). The present case of PSI is, however, quite different—inasmuch as time-resolved spectral evolution between bulk and low energy pigments is clearly observed, due to the large energy differences between these pigment pools.

The above discussion, which explains the good Kennard-Stepanov behavior of PSI-200 in terms of trapping being formally associated with the initially excited bulk antenna, would seem to exclude the possibility that the red forms are tightly coupled to the inner core pigments and hence to P700. At room temperature, trapping from the red forms in PSI-200 has recently been shown to vary between 80 and 103 ps, increasing with increasing wavelength (Jennings et al., 2003). These values correspond to trapping rates of 9.7 to 12.5 ns^{-1}. If these rates are directly associated with the red states in the model, and with the initial excitation in the bulk, the values for $\int \rho_n(t)dt$, $n = 1,4,5,6$, are no longer in agreement with the good Stepanov behavior experimentally encountered (Table 2). Thus we conclude that none of the red forms are closely coupled to the central core chlorophylls. They must therefore be distributed among the bulk pigments with energy transfer from them to the reaction center being mediated by the bulk pigments.

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